

What Is Claimed Is:

1. A vector construct consisting essentially of a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence, said vector construct further comprising an amplifiable marker.

5 2. A vector construct consisting esseintially of a transcriptional regulatory sequence operably linked to a translational start codon, a secretion signal sequence, and an unpaired splice donor site.

10 3. A vector construct consisting essentially of a transcriptional regulatory sequence operably linked to a translational start codon, an epitope tag, and an unpaired splice donor site.

4. A vector construct comprising a transcriptional regulatory sequence operably linked to a translational start codon, a secretion signal sequence, an epitope tag, and an unpaired splice donor site.

15 5. A vector construct comprising a transcriptional regulatory sequence operably linked to a translational start codon, a secretion signal secretion sequence, an epitope tag, a sequence-specific protease site, and an unpaired spice donor site.

6. The vector constructs of any of claims 2-5 containing an internal ribosome entry site for producing a polycistronic message.

20 7. The vector constructs of any of claims 2-6 further comprising an amplifiable marker.

8. The vector construct of any of claims 1-16 wherein said transcriptional regulatory sequence is a promoter.

9. The vector construct of claim 8 wherein said promoter is a viral promoter.

5 10. The vector construct of claim 9 wherein said viral promoter is the cytomegalovirus immediate early promoter.

11. The vector construct of claim 8 wherein said promoter is a cellular non-viral promoter.

10 12. The vector construct of claim 8 wherein said promoter is inducible.

13. The vector construct of any of claims 1-16 wherein said transcriptional regulatory sequence is an enhancer.

14. The vector construct of claim 13 wherein said enhancer is a viral enhancer.

15 15. The vector construct of claim 14 wherein said viral enhancer is the cytomegalovirus immediate early enhancer.

16. The vector construct of claim 13 wherein said enhancer is a cellular non-viral enhancer.

17. A cell containing any of the vector constructs of claims 1-16.

18. The cell of claim 17 in which said vector construct has integrated into the cellular genome.

19. The cell of claim 18 in which an endogenous gene is over-expressed in said cell by means of said transcriptional regulatory sequence on said vector construct.

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20. A method for making a recombinant cell comprising introducing any of the constructs of claims 1-16 into said cell.

21. A method for over-expressing an endogenous gene in a cell comprising:

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- (1) introducing any of the constructs of claims 1-16 into a cell;
- (2) allowing said construct to integrate into the genome of said cell by non-homologous recombination; and
- (3) allowing over-expression of said endogenous gene in said cell.

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22. The method of claim 21, wherein said over-expression is *in vitro*.

23. The method of claim 21, wherein said over-expression is *in vivo*.

24. The method of claim 21 wherein the expression product of said endogenous gene product is purified.

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25. A library of cells comprising a collection of cells transformed with one or more of the constructs of any of claims 1-16, wherein said constructs are integrated into the genome of said cells by non-homologous recombination, said cells over-expressing one or more endogenous genes by means of said transcriptional regulatory sequence.

26. A method of obtaining an over-expressed gene product from a library of cells comprising screening the library of claim 25 for expression of said gene product, selecting a cell from said library, said cell over-expressing said gene product, and obtaining said gene product from said selected cell.

5 27. A method for over-expressing an endogenous gene in a cell comprising:

- (1) introducing a vector comprising a transcriptional regulatory sequence into said cell;
- (2) allowing said vector to integrate into the genome of said cell by non-homologous recombination;
- (3) allowing over-expression of said endogenous gene in said cell by means of said transcriptional regulatory sequence;
- (4) screening said cell for over-expression of said endogenous gene;
- (5) culturing said cell so as to produce amounts of the expression product of said endogenous gene;
- (6) purifying said expression product.

28. A method for over-expressing an endogenous gene in a cell comprising:

- (1) introducing a vector comprising a non-retrovirus transcriptional regulatory sequence into said cell;
- (2) allowing said vector to integrate into the genome of said cell by non-homologous recombination;
- (3) allowing over-expression of said endogenous gene in said cell by means of said transcriptional regulatory sequence;
- (4) screening said cell for over-expression of said endogenous gene;

(5) culturing said cell so as to produce amounts of the expression product of said endogenous gene.

29. The method of claim 28 further comprising purifying said expression product.

5 30. A method for over-expressing an endogenous gene in a cell comprising:

(1) introducing a vector comprising a transcriptional regulatory sequence operably linked to a secretion signal sequence into said cell;

10 (2) allowing said vector to integrate into the genome of said cell by non-homologous recombination;

(3) allowing over-expression of said endogenous gene in said cell by means of said transcriptional regulatory sequence;

15 (4) screening said cell for over-expression of said endogenous gene;

(5) culturing said cell so as to produce amounts of the expression product of said endogenous gene.

31. A method for over-expressing an endogenous gene in a cell comprising:

20 (1) introducing a vector comprising a non-retrovirus transcriptional regulatory sequence operably linked to a secretion signal sequence into said cell;

(2) allowing said vector to integrate into the genome of said cell by non-homologous recombination;

25 (3) allowing over-expression of said endogenous gene in said cell by means of said transcriptional regulatory sequence;

(4) screening said cell for over-expression of said endogenous gene;

(5) culturing said cell so as to produce amounts of the expression product of said endogenous gene.

32. The method of claim 31 further comprising purifying said expression product.

5 33. A method for over-expressing an endogenous gene in a cell comprising:

(1) introducing a vector comprising a transcriptional regulatory sequence into said cell;

10 (2) allowing said vector to integrate into the genome of said cell by non-homologous recombination;

(3) allowing over-expression of said endogenous gene in said cell by means of said transcriptional regulatory sequence;

(4) screening said cell for over-expression of said endogenous gene;

15 (5) isolating and cloning said cell;

(6) allowing said cell to over-express said endogenous gene *in vivo*.

34. A method for over-expressing an endogenous gene in a cell comprising:

20 (1) introducing a vector comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence into said cell;

(2) allowing said vector to integrate into the genome of said cell by non-homologous recombination;

25 (3) allowing over-expression of said endogenous gene in said cell by means of said transcriptional regulatory sequence;

(4) screening said cell for over-expression of said endogenous gene; and

(5) culturing said cell so as to produce amounts of the expression product of said endogenous gene.

35. The method of claim 33 further comprising purifying said expression product.

5 36. A method for over-expressing an endogenous gene in a cell comprising:

(1) introducing a vector comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence into said expression cell;

10 (2) allowing said vector to integrate into the genome of said cell by non-homologous recombination;

(3) allowing over-expression of said endogenous gene in said cell by means of said transcriptional regulatory sequence;

15 (4) screening said cell for over-expression of said endogenous gene;

(5) isolating and cloning said cell; and

(6) allowing said cell to over-express said endogenous gene

in vivo.

20 37. A method for over-expressing an endogenous gene in a cell comprising:

(1) introducing a vector comprising a transcriptional regulatory sequence and an amplifiable marker into said cell;

(2) allowing said vector to integrate into the genome of said cell by non-homologous recombination;

25 (3) allowing over-expression of said endogenous gene in said cell by means of said transcriptional regulatory sequence;

(4) screening said cell for over-expression of said endogenous gene;

(5) culturing said cell under conditions in which said vector and said endogenous gene are amplified in said cell; and

5 (6) culturing said cell so as to produce the expression product of said endogenous gene.

38. The method of claim 37 further comprising purifying the product of said endogenous gene.

10 39. A method for over-expressing an endogenous gene in a cell comprising:

(1) introducing a vector comprising a transcriptional regulatory sequence and an amplifiable marker into said cell;

(2) allowing said vector to integrate into the genome of said cell by non-homologous recombination;

15 (3) allowing over-expression of said endogenous gene in said cell by means of said transcriptional regulatory sequence;

(4) screening said cell for over-expression of said endogenous gene;

(5) isolating and cloning said cell; and

20 (6) allowing said cell to over-express said endogenous gene *in vivo*.

40. The method of any of claims 20-24 and 26-48 wherein said transcriptional regulatory sequence is a promoter.

41. The method of claim 40 wherein said promoter is a viral promoter.

42. The method of claim 41 wherein said viral promoter is the cytomegalovirus immediate early promoter.

43. The method of claim 40 wherein said promoter is a cellular non-viral promoter.

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44. The method of claim 40 wherein said promoter is inducible.

45. The method of any of claims 20-24 and 26-38 wherein said transcriptional regulatory sequence is a enhancer.

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46. The method of claim 45 wherein said enhancer is a viral enhancer.

47. The method of claim 46 wherein said viral enhancer is the

cytomegalovirus immediate early enhancer.

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48. The method of claim 45 wherein said enhancer is a cellular non-viral enhancer.

49. The method of any of claims 20-24 and 26-38 further comprising introducing double strand breaks into the genomic DNA of said cell prior to or simultaneously with integration of said vector.

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50. A cell produced by the method of any of claims 20-24 and 26-38.

51. The method of any of claims 20-24 and 26-38 wherein said vector construct is linear.

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52. A method for over-expressing an endogenous gene in a cell comprising:

- (1) introducing a vector comprising a transcriptional regulatory sequence into said cell;
- (2) allowing said vector to integrate into the genome of said cell by non-homologous recombination;
- 5 (3) allowing over-expression of said endogenous gene in said cell by means of said transcriptional regulatory sequence;
- (4) screening said cell for over-expression of said endogenous gene;
- (5) culturing said cell in serum free medium.

10 53. A method for over-expressing an endogenous gene in a cell comprising:

- (1) introducing a vector comprising a transcriptional regulatory sequence into said cell;
- (2) allowing said vector to integrate into the genome of said cell by non-homologous recombination;
- 15 (3) allowing over-expression of said endogenous gene in said cell by means of said transcriptional regulatory sequence;
- (4) screening said cell for over-expression of said endogenous gene; and
- 20 (5) culturing said cell so as to produce amounts of the expression product of said endogenous gene.
- (6) purifying said expression product beginning with the cell mass equivalent of 10 liters of cells at 10^4 cells/ml.

54. A method for activating gene expression comprising:

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- (1) introducing a vector into the genome of a cell, said vector containing a regulatory sequence and unpaired splice donor site, and lacking targeting sequences,
 - (2) screening said cell for expression of a protein.

55. The method of claim 54 with the additional step of isolating the cell producing the activated protein.

56. A method for activating gene expression comprising:

- (1) integrating a vector into a cell by non-homologous recombination, said vector containing a regulatory sequence and unpaired splice donor site;
- (2) screening for nonhomologous recombinant cells that express a gene, said gene and said upstream region of said gene lacking homology to the vector.

57. A method for enhancing expression of a gene in a cell *in situ*, the phenotype of said gene being known, without making use of any sequence information of the gene, the method comprising the steps of:

- (1) constructing a vector comprising a transcriptional regulatory sequence and an unpaired splice donor sequence;
- (2) delivering copies of the vector to a plurality of cells;
- (3) culturing the cells under conditions permitting nonhomologous recombination events between the inserted vector and the genome of the cells; and
- (4) screening the recombinant cells by assay for the phenotype to identify cells in which the expression of the gene has been enhanced.

20 58. A method as claimed in claim 57 wherein the phenotype is production of a particular protein and the assay is conducted by testing for increased production of the protein.

25 59. A method for enhancing expression of a gene in a cell *in situ*, the phenotype of said gene being known, without making use of any sequence information of the gene, the method comprising the steps of:-

- (1) constructing a vector comprising a transcriptional regulatory sequence and an unpaired splice donor sequence;
- (2) delivering copies of the vector to a plurality of cells;
- (3) culturing the cells under conditions which increase the likelihood of nonhomologous recombination events between the vector and the genome of the cells; and
- (4) screening the recombinant cells by assay for the phenotype to identify cells in which the expression of the gene has been enhanced.

60. A method to activate expression of a gene in a cell *in situ* without making use of any sequence information of the gene, the method comprising the steps of:

- (1) constructing a vector comprising a transcriptional regulatory sequence and an unpaired splice donor sequence;
- (2) integrating the vector by nonhomologous recombination into at least 100,000 cells;
- (3) screening the recombinant cells by assay for the phenotype to identify cells in which the expression of the gene has been activated.

61. A purified cell comprising in its genome an inserted genetic construct, the genetic construct comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence, the construct being effective in the cell line to activate the expression of a gene, the construct inserted into a gene or upstream region of a gene, the gene and region having no homology to any sequences in the genetic construct.

62. The cell of claim 61 wherein the integrated genetic construct additionally contains an amplifiable marker.

63. A purified cell comprising in its genome an inserted genetic construct, the genetic construct comprising a transcriptional regulatory sequence operably

linked to an unpaired splice donor sequence, the construct being effective in the cell line to activate the expression of a gene, the construct containing no homology to any sequences in said gene or to upstream regions of said gene.

64. A method for activating gene expression comprising:

- 5 (1) constructing a vector comprising a transcriptional regulatory sequence and an unpaired splice donor sequence;
- (2) introducing said vector into cells;
- (3) culturing the cells under conditions permitting nonhomologous recombination events between the inserted vector and the genome of the cells;
- 10 and
- (4) screening the recombinant cells by assay for expression of a gene, said gene and upstream region of said gene having no homology to the vector.

65. A purified cell comprising in its genome an inserted genetic construct, the genetic construct comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence, said construct being effective in the cell line to activate the expression of a gene, the genetic construct inserted into a gene or upstream region of a gene by nonhomologous recombination.

66. A method for enhancing gene expression comprising:

- 20 (1) introducing a vector into the genome of a cell, said vector containing an enhancer sequence and amplifiable marker, and lacking targeting sequences,
- (2) screening said cell for expression of a protein.

67. The method of claim 66 with the additional step of isolating the cell producing the activated protein.

68. A method for enhancing gene expression comprising:

- (1) integrating a vector into a cell by non-homologous recombination, said vector containing an enhancer sequence;
- (2) screening for nonhomologous recombinant cells that express a gene, said gene and said upstream and downstream regions of said gene, in which regions the enhancer is active, lacking homology to the vector.

5 69. A method for the enhancement of expression of a gene of known phenotype in a cell *in situ* without making use of any sequence information of the gene, the method comprising the steps of:

- (1) constructing a vector comprising an enhancer;
- (2) delivering copies of the vector to a plurality of cells;
- (3) culturing cells under conditions permitting nonhomologous recombination events between the inserted vector and the genome of the cells; and
- (4) screening the recombinant cells by assay for the phenotype to identify cells in which the expression of the gene has been enhanced.

15 70. The method of claim 69 wherein the phenotype is production of a particular protein and the assay is conducted by testing for increased production of the protein.

20 71. A method for the enhancement of expression of a gene of known phenotype in a cell *in situ* without making use of any sequence information of the gene, the method comprising the steps of:

- (1) constructing a vector comprising an enhancer;
- (2) delivering copies of the vector to a plurality of cells;
- (3) culturing cells under conditions which increase the likelihood of nonhomologous recombination events between the vector and the genome of the cells; and

(4) screening the recombinant cells by assay for the phenotype to identify cells in which the expression of the gene has been enhanced.

72. A method to enhance expression of a gene in a cell *in situ* without making use of any sequence information of the gene, the method comprising the steps of:

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(1) constructing a vector comprising an enhancer;

(2) integrating the vector by nonhomologous recombination into at least 100,000 cells;

(3) screening the recombinant cells by assay for the phenotype to identify cells in which the expression of the gene has been enhanced.

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73. A purified cell comprising in its genome an inserted artificial genetic construct, the genetic construct comprising an enhancer effective in the cell line to enhance the expression of a gene, the genetic construct inserted into a gene or upstream or downstream regions of a gene, where said enhancer is effective, the gene and regions having no homology to any sequences in the genetic construct.

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74. The cell of claim 73 wherein the integrated genetic construct additionally contains an amplifiable marker.

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75. A purified cell comprising in its genome an inserted artificial genetic construct, the genetic construct comprising an enhancer effective in the cell line to enhance the expression of a gene, the genetic construct having no homology to any sequences in said gene or to upstream or downstream regions of said gene where said enhancer is effective.

76. A method for enhancing gene expression comprising:

- (1) constructing a vector comprising an enhancer;
- (2) introducing said vector into cells;

(3) culturing cells under conditions permitting nonhomologous recombination events between the inserted vector and the genome of the cells; and

5 (4) Screening the recombinant cells by assay for expression of a gene, said gene and upstream and downstream regions of said gene, where said enhancer is effective, having no homology to the vector.

77. A purified cell comprising in its genome an inserted genetic construct, the genetic construct comprising an enhancer effective in the cell line to activate the expression of an endogenous gene in said cell, the genetic construct inserted into 10 a gene or upstream or downstream region of a gene by nonhomologous recombination.